

# Genetic variability of pedunculate oak in the Bosnian provenance test

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## Introduction

Pedunculate oak (*Quercus robur* L.) used to be an economically important type of Bosnia and Herzegovina forests, but according to the data from the Bosnia and Herzegovina forest inventory on big areas in 1964 – 1968 (Matić et al. 1971), only cca 10,261 ha is accounted for pedunculate oak forests.

Area of Bosnia and Herzegovina represents the central part of natural southern spread of pedunculate oak and as such it has a specific genetic structure (Ballian et al. 2010) in relation to the northern and central part of the areal.

So far, very few pedunculate oak molecular researches have been conducted in Bosnia and Herzegovina. Ballian et al. (2010) have tested the variability of certain remaining populations of pedunculate oak in Bosnia and Herzegovina, using high polymorphic nuclear microsatellite markers. They have recorded significant differences in frequencies between populations, effective number of alleles, fixation index and genetic differentiation. Memisevic Hodzic (2015) have tested twenty pedunculate oak provenances using isozyme markers. The analysis has proven that there exist massive variabilities within and between the populations.

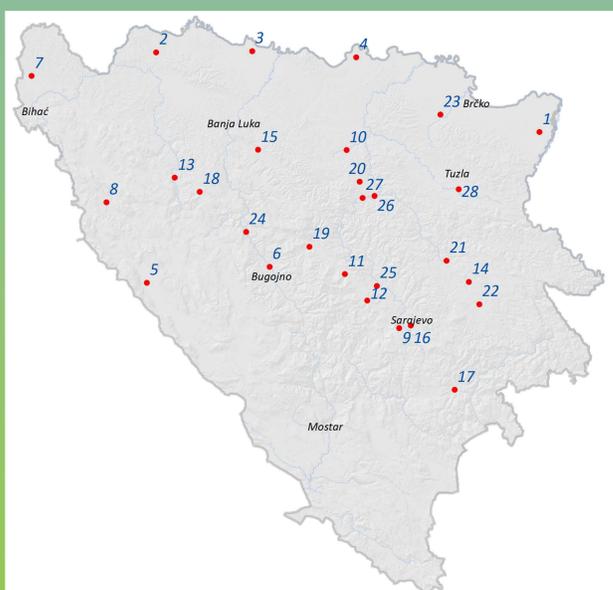
The aim of this study is analyse pedunculate oak provenances and to provide answers to questions about the genetic structure of the remaining populations on the territory of Bosnia and Herzegovina, whether it is possible to provide predispositions for future production of seeds and planting material and the experimental re-ionisation of pedunculate oak in Bosnia and Herzegovina, i.e. conservation *in situ* and *ex situ*.

## Material and methods

Research has been conducted in provenance test of pedunculate oak originating in Bosnia and Herzegovina. The test has been taken from the seeds in natural populations, gathered under minimum 10 trees per population. Twenty provenances have been analysed in total (Table 1, Map 1). Twigs with dormant buds were collected in February 2014. Fifty plants were taken from each provenance.

Table 1: Analysed pedunculate oak provenances

No.	Provenance	North latitude	East longitude	Altitude	Number of analysed plants
1	Bijeljina	44° 43' 50"	19° 13' 30"	93	50
2	Bosanska Dubica	45° 06' 24"	16° 40' 32"	145	50
3	Bosanska Gradiška	45° 06' 64"	17° 18' 63"	91	50
4	Bosanski Brod	45° 05' 27"	18° 00' 38"	84	50
5	Bosansko Grahovo	44° 01' 05"	16° 38' 24"	703	50
6	Bugojno	44° 06' 00"	17° 26' 31"	537	50
7	Drvar	44° 23' 39"	16° 21' 54"	462	50
8	Hrgovi Srebrenik	44° 49' 06"	18° 34' 11"	133	50
9	Jelah	44° 39' 09"	17° 56' 46"	181	50
10	Kačuni	44° 03' 59"	17° 56' 13"	443	50
11	Ključ	44° 30' 56"	16° 48' 42"	260	50
12	Kotor Varoš	44° 39' 07"	17° 21' 35"	252	50
13	Miljevina Foča	43° 31' 06"	18° 38' 56"	627	50
14	Mrkonjić Grad	44° 27' 04"	16° 58' 42"	753	50
15	Mutnica Cazin	44° 58' 55"	15° 50' 54"	270	50
16	Olovo	44° 07' 44"	18° 36' 11"	542	50
17	Sokolac	43° 55' 17"	18° 48' 53"	866	50
18	Stojčevac Ilidža	43° 48' 40"	18° 17' 25"	506	50
19	Žepče	44° 25' 35"	18° 03' 10"	224	50
20	Živinice	44° 27' 58"	18° 41' 09"	216	50
Total					1000



Map 1: Investigated provenances

Isozyme markers have been used for the analysis of genetic structure (Table 2), while the interpretation of zymogram has been conducted in accordance with the Konner et al (2004) protocol. *Statistical Analysis System* has been used for calculating parameters of genetic variability.

Table 2: Enzyme systems used, analyzed gen loci

No	Enzyme systems	Analysed genetic loci
1.	Alaninaminopeptidase	AAP-A
2.	Aspartataminotransferaze	GOT-B
3.	Fluoreszierende Esterase	FEST-A, FEST-C
4.	Alkoholdehydrogenase	ADH-A
5.	Isocitratdehydrogenase	IDH-A, IDH-B, IDH-C
6.	Menadionreduktase	MNR-A
7.	6-Phosphogluconatdehydrogenase	6-PDGH-A, 6-PDGH-B
8.	Phosphoglucose-isomerase	PGI-B
9.	Phosphoglucomutase	PGM-B
10.	Shikimatdehydrogenase	SKDH-A

## Results and discussion

### Intraprovenance variability

#### Number of alleles per locus

The lowest detected average value of number of alleles per locus (Table 3) have Bosanska Dubica, Grahovo, Kotor Varos, Olovo and Stojčevac provenances (2,3571), while the highest value is detected in Bosanski Brod provenance (3,1429).

#### Loci polymorphism

The lowest percentage of polymorphic loci is found in Bosanska Dubica, Mutnica and Stojčevac provenances with 50%, while the greatest percentage is in Zivinice provenance, 85,71% (Table 3).

#### Observed heterozygosity

As shown in Table 3, the highest value of observed heterozygosity is found in Olovo provenance (0,2907), while the lowest value is found in Bosanska Dubica provenance (0,1571).

Observed heterozygosity is greater than expected for the following eight provenances: Bosanski Brod, Hrgovi Srebrenik, Jelah, Kačuni, Kotor Varos, Olovo, Stojčevac and Zepce. This result had been expected for Bosanski Brod provenance which is open to Posavina populations in Croatia, as well as for Hrgovi Srebrenik, Jelah, Kotor Varos, Kačuni and Zepce populations which still have good structures and which are relatively large in comparison to other populations in Bosnia and Herzegovina.

#### Fixation index (Wright's inbreeding coefficient)

Eight provenances show negative average values of the fixation index. The highest value of fixation index are found in Bosanska Dubica (0,1019), and the lowest in Olovo provenance (-0,1269). In this population one can detect the phenomenon of a small population and a great diversity. Average fixation index value for all provenances, as well as for genetic loci, is positive, amounting to 0,0032.

Table 3: Measures of genetic variability for the 20 analysed provenances

Provenance	Average Allele/Locus	Polymorphic loci (%)	Observed heterozyg.	Theoretic heterozyg.	Fixation index (Fi)
Bijeljina	2,9286	64,29	0,2208	0,2375	0,0319
Bosanska Dubica	2,3571	50,00	0,1571	0,2028	0,1019
Bosanska Gradiška	2,7857	64,29	0,2059	0,2107	-0,0144
Bosanski Brod	3,1429	64,29	0,2611	0,2608	-0,0104
Bosansko Grahovo	2,3571	57,14	0,1896	0,2246	0,0703
Bugojno	2,4286	64,29	0,2309	0,2324	-0,0175
Drvar	3,0714	64,29	0,2443	0,2605	0,0927
Hrgovi Srebrenik	2,7857	71,43	0,2427	0,2414	-0,0085
Jelah	2,6429	64,29	0,2843	0,2804	0,0090
Kačuni	2,7857	71,43	0,2617	0,2569	-0,0150
Ključ	2,8571	57,14	0,2397	0,2479	-0,0010
Kotor Varoš	2,3571	78,57	0,2392	0,2294	-0,0242
Miljevina	3,0714	71,43	0,2165	0,2519	0,0808
Mrkonjić Grad	2,7143	64,29	0,2102	0,2264	0,0163
Mutnica	2,7857	50,00	0,2008	0,2046	-0,0150
Olovo	2,3571	64,29	0,2907	0,2388	-0,1269
Sokolac	2,7857	71,43	0,2489	0,2551	0,0027
Stojčevac	2,3571	50,00	0,2190	0,1867	-0,0566
Žepče	2,5714	57,14	0,2106	0,1983	-0,0467
Živinice	2,7857	85,71	0,2690	0,2731	-0,0034
Average	2,6964	63,93	0,2322	0,2360	0,0032

### Differentiation between provenances

#### Multilocus divergence between the provenances

The lowest mutual multilocus divergence is detected between Bijeljina and Mrkonjić Grad provenances, amounting to 0,10006, while the highest divergence is 0,3217, detected between Olovo and Kotor Varos.

#### Genetic pool differentiations between provenances

The lowest mutual divergence of the genetic pool is detected between Bosanska Gradiska and Stojčevac provenances, amounting to 0,0560, while the highest divergence is found between Olovo and Zepce provenances, amounting to 0,2066. Low divergence between Bosanska Gradiska and Stojčevac populations was not expected because they are geographically distant.

## Conclusions

- Through the analysis of ten enzyme systems with 14 genetic loci we determined the existence of statistically significant differences between the analysed provenances.
- Each of the 14 analysed loci is more or less polymorphic. A high level of polymorphism is found in AAP-A locus.
- The greatest number of polymorphic loci is found in Zivinice provenance (12).
- The lowest determined average value of number of alleles is detected in Bosanska Dubica, Bosansko Grahovo and Sokolac provenances, while the greatest value is in Bosanski Brod provenance.
- The highest average observed heterozygosity for all genetic loci is shown by Olovo provenance, while the lowest is shown by Bosanska Dubica.
- Positive average values of fixation index have been determined in eight out of twenty provenances: Bijeljina, Bosanska Dubica, Bosansko Grahovo, Drvar, Jelah, Miljevina, Mrkonjić Grad and Sokolac.
- Minimum multilocus diversity has been established between Bijeljina and Mrkonjić Grad provenances, while the maximum multilocus diversity has been established between Olovo and Kotor Varos provenances.
- Minimum diversity of the genetic pool has been established between Bosanska Gradiska and Stojčevac provenances, while maximum diversity of genetic pool has been established between Olovo and Zepce provenances.
- The obtained results should be used during planning measures of preservation and reintroduction of pedunculate oak in Bosnia and Herzegovina and for management activities in the remaining stands.

## References

- Ballian, D., Belletti, P., Ferrazzini, D., Bogunic, F., Kajba, D. 2010: Genetic variability of Pedunculate oak (*Quercus robur* L.) in Bosnia and Herzegovina, *Periodicum Biologorum* 112 (3): 353–362
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